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54 **A method for forming ultra-fine structures.**

57 The present invention provides a method for forming ultra-fine structures comprising the steps of binding atoms or molecules to at least amino end or carboxyl end, or in the vicinity thereof, of flagellin, subunit or a bacterial flagellum, and polymerizing these flagellins.

These ultra-fine structures are useful for the three-dimensional wiring or connectors of micro electronics circuit as covered micro conductors, as well as for micromachines as various functional materials.

## [FIELD OF THE INVENTION]

This invention relates to a method for forming ultra fine-structures. More specifically, it relates to a method for forming ultra-fine structure which are useful for the connecting wires or connectors of micro electronic circuits as covered conductors, and which are also usable as ultra-micro functional materials.

## [PRIOR ART ]

Dramatic progress in electronics engineering during recent years, represented by the super computer, has made possible the high-speed processing of information which was heretofore impossible. This helps achieve the provision of more precise weather information, greater precision and higher degree in ultra LSI designs, and the development of innovative materials. To perform large-scale and highly precise calculations enough to be put to a practical application, however, computers operating at a speed several tens of times as high as that available at present are required, and for this purpose, at ultra-high speed operators and large-scale memories are necessary.

On the other hand, when we consider the signal transmission speed within a computer, even light, which travels the the fastest of all, can travel only 3 centimeters in 1/10 billion of a second anticipated to be speed of future ultra-high speed computers. To enhance the hardware performance of the computer, therefore, it is indispensable to miniaturize the system and construct it in higher density, as well as to make the circuit devices operate at higher speeds.

From such viewpoint, the electronics circuit presently available is being downsized from micron to submicron unit: such downsizing is expected to be further proceeding. In keeping up with this trend, downsizing is required of not only individual circuit devices, but also the wires used to connect the same.

Furthermore, a liquid crystal display (LCD) is, for example, becoming increasingly compact and lightweight, since liquid crystal is much thinner and lighter in weight than its similar devices, and very small voltages at which it is operated and driven are required.

Nevertheless, when we seek to further downsize the LCD, it becomes difficult to maintain the qualities of the screen (e.g., contrast and visibility) to high level. When a character is displayed on an LCD screen, such as LCD TV receiver, each LCD is normally driven by a method known as multi-matrix electrode system. In order to increase the display capacity by N times by this method, the driving circuit and terminal connections N times as many are required.

For this reason, to achieve the downsized system and increased display capacity, it becomes indispensable to make LCD driving electrode structure and electronics circuits more refined and finer, and also to downsize the connectors connecting therebetween to a finer size.

It is exceedingly difficult, however, to make the present microelectronics circuits much finer and more integrated. For instance, the wires of circuits, such as deposited metallic membrane used in semiconductor chips, are not insulated; hence this uncovered wire not only requires the utmost care in handling, but also is essentially unsuited to three-dimensional wiring. For connecting liquid crystal devices and driving electronics circuits, pin connectors, elastic connectors or flexible connectors, for instance, are employed. These connectors are provided with only dozens of covered conductors per 1 mm, and even with semiconductor technologies, this conductor is in submicron units at the utmost. Under these circumstances, interest has been mounting in constructing biochip and biocircuits utilizing the intergration of proteins and functions of microorganisms.

Approaches to ultra-refinement and ultraintegration using these biodesign technologies are being noted in the medical and micromachine fields.

However, under the present circumstances, virtually no specific means to develop these biodesign technologies have been established. For instance, how living matter and organic molecules will be specifically used in a given location or structure remains to be known.

## [SUMMARY OF THE INVENTION]

The present invention has the objective of providing a method for forming ultra-fine structures which are useful as ultra-micro conductors which permit micro and high density wiring of micro electronics circuits, or ultra-fine functional materials necessary for the realization of micromachines.

The present invention provides a method for forming an ultra-fine structure comprising the steps of bonding atoms or molecules at least amino end of carboxyl end, or in the vicinity thereof, of flagellins constructing a bacterial flagellum, and polymerizing the flagellins.

This invention also provides a method for forming an ultra-fine structure in which the polymerized flagellin prepared by the foregoing method is oriented in a magnetic field.

## [BRIEF DESCRIPTION OF THE DRAWING]

FIG. 1 indicates a front sectional view of the bacterial flagellum used to form ultra-fine structures according to the present invention. The white-on-black portion in the figure illustrates the shape of flagellin, a subunit of the flagellum, and each of the domains thereof (D1, D2 and D3).

## [DETAILED DESCRIPTION OF THE INVENTION]

On the following pages, detailed descriptions will be described on the principle and construction of the present invention.

The ultra-fine structure according to the present invention is featured by being prepared using the characteristics unique to a flagellin, a subunit of a bacterial flagellum, i.e., self-assembly, amino acid sequence characteristics.

Flagellum is a helical filament consisting of single kinds of proteins known as flagellin, possessing a 6-nm-diameter central hole passing therethrough. The whole diameter thereof is as fine as approx. 25nm, but the flagellum is not limited in dimensions in the lengthwise direction, normally extending several times (10 - 20 $\mu$ m) of its bacterial body.

The inventors of the present invention has discovered that the flagellin consists of three domains (D1, D2, D3) as indicated in FIG. 1, and that the D1 domain, located in the central core part and forming a central Channel is involved in a self-assembling formation of the flagella. Correlations between each domain and amino acid sequences revealed that the D1 domain consists of the ends of a amino acid sequence of flagellin, that is to say, the amino end and the carboxyl end.

The present invention has been made on the basis of the aforesaid very significant knowledge.

Flagellum is re-constructed by monomerizing the flagellins of the flagellum, chemically bonding atoms or molecules of various metals, semi-metals and non-metals to the amino or carboxyl end of the D1 domain through covalent bonding, hydrogen bonding, etc., then by polymerizing these flagellins in a self-assembling manner. As a result, the thus re-constructed flagellum has a band consisting of these atoms and molecules on the inside peripheral surface thereof. When using conductive metals as atoms, for instance, a conductive band is formed. This conductive band is completely covered with flagellins, insulators, and hence an covered conductive ultra-micro structure with scores of  $\mu$ m long and with a diameter of approx. 20 nm can be prepared. When photo-responsive molecules are used, ultra-fine structure which consist of photo-responsive functional band regions is formed.

Of the domains constituting flagellins, the amino acid sequence in D1 domain is virtually in common with those in comprehensive kinds of bacteria. Accordingly, flagellins of a wide variety of bacteria can be used in this invention. Since the flagellins of salmonella strains are readily available and excel in linearity, they are especially preferred for the present invention.

Then, descriptions will be given more specifically about a method for forming ultra-fine structures according to the present invention, together with examples.

## (1) Preparation of flagellines

Flagellins, a subunit of flagellum, can be synthesized by genetic manipulation, and also can be extracted and prepared from the flagellum of bacterias by the ordinary method. For instance, the cultured bacteria is subjected to shear force, which is created in a buffer liquid consisting of Tris 20 mM (pH 7.8) + NaCl 0.15M. Then the flagella are collected by centrifugal separation. Then, these flagella are heat-treated at 65°C for ten minutes, providing a flagellins as a simple substance of monomerized flagella.

## (2) Binding of atoms or molecules

Atomic or molecular compounds are caused to be bound to the amino end or carboxyl end of proteins which constitute the D1 domain of individual flagellin through a known chemical reaction.

In this case, there are no special limitations to the type of atoms or molecules. Metallic, semiconductor or non-metallic atoms, or photo-conductive, magnetic-conductive and other functional organic or inorganic compounds can be employed. These atoms or molecules can be chemically bonded to the amino end or carboxyl end through covalent bond or hydrogen bond.

For example, more specifically, metallic or semiconducting atoms or molecules may be bound to amino acid residues on the wall surface of the central hole of the flagellum, and the following bindings can be exemplified:

Hg (mercury) : binding to Arg (arginine), Cys (cysteine) and the like.

Ag (silver) : binding to His (histidine).

Sm (samarium) : binding to Glu (glutamic acid).

Other Pt, Au, and Pd are adhered to amino acid residues.

Silicon and germanium can be conceived as semiconductors. They can be introduced while silicon is allowed to be covalently bonded with the sulfur in cysteine residue or methionine residue since it is difficult for them to be bound to amino acid as they are.

As for nonmetal, a polymer to be crosslinked with ultraviolet rays, for example, can be bound to the amino end or carboxyl end of the flagellin.

### (3) Reconstruction of flagellum

Ammonium sulfate is added to a flagellin solution in which each flagellin binds atom or molecule, causing the flagellin to be polymerized in a self-assembling manner and flagella to be reconstructed.

In this case, the length of the flagella depends upon the density of flagellins in a buffer liquid and upon the density of ammonium sulfate to be added thereto. For instance, when 1M ammonium sulfate is added to 3 to 10 mg/ml flagellin solution, approx. 0.5  $\mu$ m flagellum is formed in several minutes. However, if the ammonium sulfate is 0.5M and the flagellin is 1mg/ml or less, it takes more than 10 hours to form 10 to 100  $\mu$ m flagellum. Accordingly, it is preferred to add 0.5 to 2M ammonium sulfate to 1 to 10 mg/ml flagellin solution.

Lastly, ultra-fine structures having the specified atoms or molecules continuously on the wall surface of the central hole of the flagellum is completed by using Tris 20 mM (pH 7.8) + NaCl 0.15M, Gly 0.2M (pH 8.0) and the like as buffer liquid.

If conductive metal is bound to the central hole of such ultra-fine structure, the flagellar protein becomes an insulator, and hence this structure becomes an ultra-fine covered electrical wire which conducts electric current in its center alone. Since this electrical wire is completely covered, it can be used in any shape as covered wire. Especially if flagellins over which metal is bound is introduced at given intervals during the polymerization, the resulting structure becomes wire having an electrode at given intervals. If this covered conductive ultra-fine structure is brought into direct contact with metal, tunnel current flows, forming a Josephson device.

Moreover, the ultra-fine structures formed by the foregoing process can have the atoms or molecules bonded to the central hole thereof bonded or crosslinked with each other by adding thermal or optical energy thereto. For instance, a flagellum polymers to which nonmetal polymers are bound become spiral-shaped ultra-fine line structures by crosslinking each of the polymers. Hence, this can be used directly as ultra-fine structured functional materials for springs.

Ultra-fine line structures in which the atoms or molecules in the central holes are bonded or crosslinked with one another in this way can decompose or eliminate the flagellins constituting the surroundings thereof by means of heating. By doing this, it is possible to form further ultra-fine structures which consist only of continuous atoms or molecules.

Then, detailed descriptions will be given as to a method for forming another ultra-fine structures according to the present invention.

This method forms ultra-fine structures using the magnetic configuration of the flagellum, which the inventors of this invention found, together with the aforesaid self-assembly and amino acid sequence characteristics thereof.

On the following descriptions, an example will be given as to a method for forming one-dimensional conductor in which ultra-fine covered conductors are arranged parallel in ultra-fine pitches on the substrate.

Each ultra-fine covered conductor uses flagella to whose inner wall surface conductive materials are bonded. For this flagellum, the one of salmonella, particularly the linear mutant is desired. These flagella are dissolved in several percents in Gly 0.2M (pH 8.0) + 10 mM ammonium sulfate, and this solution is titrated onto the substrate. If this substrate is located in a magnetic field so that the surface onto which the solution is titrated becomes parallel therewith, each flagellin to which conductive materials are bound is oriented in parallel with the direction of magnetic field as an axis, due to its magnetic configuration. Lastly, if the solution is allowed to dry, one-dimensional conductor of ultra-fine pitches is formed on the substrate. If it is allowed to dry, the operation is performed so that it will take the solution about one hour to become dried.

The one dimensional conductor thus formed has each conductor thereof covered with proteins. And hence, if the end is press-fit, an electronic junction with electrode terminals can be readily obtained, and for example, can be used as connectors of fine electronic circuits.

**[EFFECTS OF THE INVENTION]**

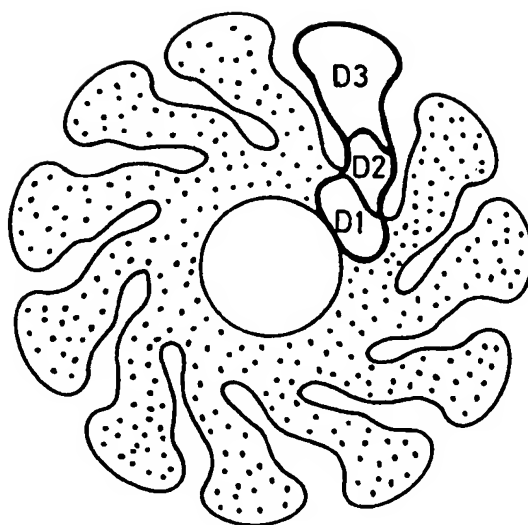
As has been described in detail above, the present invention provides ultra-fine structures which utilize bacterial flagella, as one embodiment of the bio-design technology.

These ultra-fine structures are useful for the three-dimensional wiring or connectors of micro electronics circuit as covered micro conductors, as well as for micromachines as various functional materials.

**Claims**

1. A method for forming an ultra-fine structure, which comprises binding atoms or molecules to at least the amino end or the carboxyl end or the vicinity thereof of one or more flagellins, and assembling a number of said flagellins to form said structure.
2. A method according to claim 1, wherein the assembled flagellins are heated or irradiated to cause them to be bonded or crosslinked to one another.
3. A method according to claim 2, wherein the flagellins are liberated from assembled flagellins which are bonded or crosslinked to one another.
4. A method for forming ultra-fine structures, which comprises binding atoms or molecules to at least the amino end or the carboxyl end or the vicinity thereof of flagellins assembling said flagellins to form said structure, and causing the assembled flagellins to be oriented in one direction in a magnetic field.
5. A method according to claim 1 and claim 4, wherein the bacterial flagellum is a sub-unit of a bacterial flagellum.
6. A method as claimed in claim 5 in which the flagellum is derived from a salmonella species.
7. A method as claimed in any of the preceding claims in which the atoms or molecules bound to said amino or carboxyl ends form an electrically conductive filament.
8. Flagellin molecules having atoms or molecules attached to at least the amino end or the carboxyl end or the vicinity thereof and being assembled into an ultrafine structure.
9. A method for forming an ultra-fine structure, which comprises binding atoms or molecules to one or more flagellins so that a flagellum-like structure is formed upon assembly of the flagellin.
10. Flagellin molecules having atoms or molecules attached thereto so that a flagellum-like structure is formed upon assembly.
11. An array of flagellum-like structures comprising flagellin molecules having atoms or molecules attached thereto.

*FIG. 1*





European Patent  
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# EUROPEAN SEARCH REPORT

Application Number

EP 91 31 2089

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A	NATURE, vol. 342, no. 6250, 7 December 1989, LONDON GB pages 648 - 654; K. NAMBA ET AL.: 'STRUCTURE OF THE CORE AND CENTRAL CHANNEL OF BACTERIAL FLAGELLA,' * the whole document *	1-11	C07K13/00 H01B1/20 H01B1/22 H01B1/24 H01B3/18
A	WO-A-8 808 875 (THE WASHINGTON TECHNOLOGY CENTER ) * claims *	1-11	
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)
			C07K H01B
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 08 APRIL 1992	Examiner RYCKEBOSCH A.O.
<p><b>CATEGORY OF CITED DOCUMENTS</b></p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons</p> <p>*****</p> <p>&amp; : member of the same patent family, corresponding document</p>			

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